Mycorrhizal formation by various carnivorous plants

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Introduction

Carnivorous plants receive the greatest amount of attention for their modified leaves used for obtaining extra nutrients while they live in low-nutrient soils (Slack 2000). Another way in which plants obtain more nutrients when nutrients in the soil are limiting is the formation of mycorrhizae with fungi (Nemec 1982). The fungi provide a large, highly-absorptive surface area for the plants, able to scavenge rare nutrients efficiently, while the plants provide their fungal partners with carbohydrates. This mycorrhizal habit is found in many forest trees and also in most heaths and in some epiphytes and orchids (Marx 1982; Lambers & Colmer 2005). More and more plants are being discovered to be mycorrhizal (Lambers & Colmer 2005).

With this effect of enhancing the uptake of nutrients from such a relationship, carnivorous plants might be expected to form mycorrhizae, but there are very few reports of such symbioses among carnivores, or even among their fellow wetland plants in general, with one recent report in *Drosera* a rare exception (Fuchs & Haselwandter 2004). Also, Venugopal & Raseshowri (2007) have recently reported the formation of mycorrhizal associations by *Drosera peltata* which actually uses modified underground stems and leaves in place of roots for their connections with mycorrhizal fungi. This contradicts the common belief that there is no association of mycorrhizal fungi with roots of either hygrophylic or xeromorophic carnivorous plants (MacDougal 1899, reviewed by Juniper *et al.* 1989).

In our study, 23 species of greenhouse-grown carnivorous plants from various genera were examined to identify the extent of mycorrhizal colonization and the degree of mycorrhizal development within their root systems.

Materials and Methods

Plants that were used during this study were greenhouse grown in Corydon, Indiana, USA. They were propagated either from seed or from greenhouse-grown vegetative fragments. Plants were not inoculated with mycorrhizal spores. Thus, any mycorrhizae found would have come from the potting materials used, or from other environmental sources in the greenhouse.

Twenty three species were examined in total. A representative sample of different parts of the root (or stolons, for rootless species) from each species was obtained and preserved in 95% ethanol for a minimum of 24 hr. Individual root samples of 1-2 cm were placed on a slide, washed with deionized water and stained using a mixture of 80% (by volume) solution A, and 20% (by volume) solution B. Solution A was 0.3% (by mass) aniline blue in 90% ethanol. Solution B was Lactophenol Blue Solution (FLUKA, Fuchs, Switzerland; Marx 1982; Nemec 1982; Ruzin 1999).

Three 1-2 cm sections of root or stolons were selected randomly from samples of each spe-

Table 1: Mycorrhiza-formation scores (VAM=Vesicular Arbuscular Mycorrhizae)		
Taxon	Root system	Score (0 - 10)
Nepenthaceae		
Nepenthes sanguinea	Weak/medium	4
Lentibulariaceae		
Pinguicula laueana	Weak, and large	6
Pinguicula moranensis	Weak	5
Pinguicula planifolia	Weak, and large	5
Utricularia cornuta	None	0
Utricularia inflata	None	6
Utricularia longifolia	None	2
Utricularia nephrophylla	None	0
Utricularia paulineae	None	3
Utricularia striata	None	0
Sarraceniaceae		
Sarracenia purpurea	Weak/medium	2-3
Sarracenia psittacina	Weak/medium	4
Sarracenia rubra subsp. *gulfensis*	Weak/medium	6
Darlingtonia californica	Weak/medium	7
Droseraceae		
Dionaea muscipula	Weak, but fleshy	4
Drosera adelae	Weak, and large	3
Drosera androsacea	Weak, and large	4
Drosera aliciae	Weak, and large	7
Drosera binata	Weak, and large	6
Drosera patens × *occidentalis*	Weak, and large	3
Drosera scorpioides	Weak, and large	3
Drosera omissa	Weak, and large	5
Drosera prolifera	Weak	3, mostly VAM
Drosera aliciae	Weak, and large	0 (Control)
Drosera patens × *occidentalis*	Weak, and large	0 (Control)

cies and used for staining and analysis. Sections were stained for approximately 30 min, with the exceptions of *Drosera binata* and *Nepenthes sanguinea*, both of these plants having a thick, dark root structure. Their root sections were stained for less than 2 min to allow some detail to be seen after staining and then washed thoroughly, like other samples, with deionized water to remove the excess stain. A cross-section cut from the sample was used in the situation when entire root was too thick or too dark to be viewed under the microscope. After being washed with deionized water for approximately 5 min to remove excess stain the root samples were ex-

amined under a compound microscope. Fungal colonization was measured using the following numerical scale devised by Moberly & Darnowski (unpublished):

0: No staining or staining of bodies clearly identified as structures other than mycorrhizae.
1: 1-3 cells in the field of view with mycorrhizal infection.
2: 4-5 cells in the field of view with some mycorrhizal infection or a similar number or bodies or mass of bodies in a smaller number of cells in the field of view.
3: 6-8 cells in the field of view with some mycorrhizal infection or a similar number or bodies or mass of bodies in a smaller number of cells in the field of view.
4: 9 or more cells in the field of view with some mycorrhizal infection or a similar number or bodies or mass of bodies in a smaller number of cells in the field of view.
5-10: Multiple clusters of cells scoring as in 1-4 above, with the total score for all clusters in the field of view being 5-10.

In using the above ranking scheme, more elaborately detailed or larger mycorrhizal clusters in a given cell raised the score by 1. As negative controls, we used roots from *Drosera aliciae* and *Drosera patens* × *occidentalis* (*i.e., Drosera* sp. "Lake Badgerup") that were grown in aseptic culture media at the lab.

Results

Table 1 illustrates the results of this survey on the presence of mycorrhizal colonization. All but three of the 23 species examined, the exceptions being *Utricularia striata*, *Utricularia cornuta*, and *Utricularia nephrophylla* (all bladderworts and the first and third aquatic plants), formed mycorrhizae when grown in the greenhouse. Wide variation in the shapes and abundance of mycorrhizae was observed in different species. *Drosera aliceae*, *Drosera scorpioides*, *Darlingtonia californica*, and *Sarracenia rubra* subsp. *gulfensis* formed a very large number of fungal colonizations, while other species, such as *Sarracenia purpurea*, *Utricularia longifolia*, and *Utricularia paulineae* formed very few associations.

Discussion

Since the observations of Nitschke (1860) and Burbidge (1897), there has been a general belief that carnivorous plants only have weakly developed roots, *e.g.*, most *Drosera* species, *Dionaea*, *Pinguicula*, *Cephalotus*, *Sarracenia*, and most *Nepenthes*, or no roots at all, *e.g.*, *Utricularia*, *Genlisea*, and *Aldrovanda*. The absence of roots does not necessarily mean, however, that the functions or roots are not needed; in some plants the stem and leaves have replaced their functions (Lambers & Colmer 2005). Therefore, mycorrhizal associations might occur, even in carnivorous plants where true roots have been replaced by modified shoot parts. In particular, this applies to species of *Utricularia*, and as can be seen in Table 1, even some aquatic *Utricularia* showed signs of fungal associations. Given that, it is not surprising that terrestrial and epiphytic species that grow in moist soil and in decomposing organic matter from other plants (Taylor 1989) also often showed signs of mycorrhizal infection.

On the basis of a recent ecophysiological study (Adamec 2005) carnivorous plant roots appear to be physiologically very active per unit biomass, in spite of their relatively weak proportion of total plant biomass. Since the prevailing amount of mineral nutrients in carnivorous plants gained by roots, the activity of which is greatly stimulated by foliar nutrient absorption from prey, the role of roots is crucial also for carnivory (Adamec 2005), making the general presence of mycorrhizae in cultivated carnivorous plants sensible. Further study of wild populations is needed to confirm the presence of mycorrhizae in natural growth conditions.

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